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REVIEW ARTICLE

Hyperleukotrieneuria in Patients with Allergic and Inflammatory Disease

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ABSTRACT

Cysteinyl leukotrienes (CysLTs: leukotrienes C_4, D_4, and E_4) have long been implicated in the pathogenesis of asthma and several allergic diseases. LTE_4 has been identified as a major metabolite of LTC_4, and urinary LTE_4 ($U\text{-}LTE_4$) is considered as the most reliable analytic parameter for monitoring the endogenous synthesis of CysLTs. From recent studies on the $U\text{-}LTE_4$ associated with adult stable asthma we identified four factors for hyperleukotrieneuria, namely, aspirin intolerance, eosinophilic nasal polyposis (ENP), vasculitis, and severe asthma. In ENP, there is prominent infiltration of eosinophils in the sinus and polyp tissues, which is linked to adult asthma and aspirin sensitivity, and ENP is the most important factor for the overproduction of CysLTs in asthmatics. We also demonstrated that anaphylaxis and eosinophilic pneumonia (EP) are associated with a marked increase in the $U\text{-}LTE_4$ concentration. Under these disease conditions, $U\text{-}LTE_4$ may be one of the candidate biomarkers. Moreover, the changes in $U\text{-}LTE_4$ concentrations may provide valuable information concerning therapeutic targets.

KEY WORDS

anaphylaxis, aspirin-intolerant asthma (AIA), Churg-Strauss syndrome (CSS), cysteinyl leukotrienes (CysLTs), nasal polyp

STABLE ASTHMA WITH HYPERLEUKOTRIENEURIA

Leukotrienes (LTs) are downstream products of the metabolism of cell or nuclear membrane phospholipids. In certain inflammatory cells such as eosinophils, mast cells, and other inflammatory cells, degradation to arachidonic acid (AA) by phospholipase A_2 occurs at the nuclear membrane, which indicates the biologic functions of the LTs once they are formed.[1-4] 5-lipoxygenase activation at the cytoplasmic or nuclear membrane then leads to the production of an unstable intermediate known as leukotriene A_4 (LTA_4), which can be further metabolized, depending on cell type, to LTB_4 or the cysLTs (LTC_4, LTD_4, and LTE_4). LTC_4 synthase metabolizes LTA_4 to LTC_4 via glutathione transferase. LTC_4 is then rapidly metabolized to LTD_4 and LTE_4 through the enzymes gamma-glutamyl transpeptidase and a dipeptidase. LTC_4 and LTD_4 both have very short half-lives, whereas LTE_4 appears to be the most stable of the three, with the longest half-life.[5]

LTE_4 has been identified as a major metabolite of LTC_4; urinary LTE_4 ($U\text{-}LTE_4$) has been considered as the most reliable analytic parameter for monitoring the endogenous synthesis of CysLTs.[6] We previously described an efficient procedure for the precise quantitation of LTE_4 in a small volume of urine, which was achieved mainly by the use of an Empore extraction disk cartridge.[7] Asano *et al.* reported that no systematic variation in urinary LTE_4 excretion rates over the course of a day was observed in either normal subjects or patients with stable asthma.[8] Even under a clinically stable condition, the $U\text{-}LTE_4$ concentration in patients with aspirin-intolerant asthma (AIA) is significantly higher than that in patients with aspirin-tolerant asthma (ATA) (Fig. 1).[9-12] We evaluated the clinicopathological factors associated with the increase in $U\text{-}LTE_4$ concentration in asthmatics and have confirmed that the $U\text{-}LTE_4$ concentration in AIA patients is significantly higher than those in ATA patients. Depending on asthma severity, ATA patients with severe asthma and AIA patients with poor pulmonary function showed a significant increase in $U\text{-}LTE_4$ concentration, but the extent of increase was not large (Fig. 2).[11] Moreover, we have demonstrated

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for the first time that nasal polyposis is one of the most important factors that indicate hyperleukotrienuria.[11] Recently, we have determined another clinicopathological factor for hyperleukotrienuria in patients with adult asthma. Churg-Strauss syndrome (CSS) is characterized by the presence of asthma, eosinophilia, and small-vessel vasculitis with granuloma.[13,14] The natural history of CSS is, first, the appearance of eosinophilic rhinosinusitis, followed several years later by the development of difficult asthma with marked peripheral blood eosinophilia, and finally the development of systemic vasculitis.[13] We have demonstrated that the U-LTE$_4$ concentration is elevated in the acute phase of CSS. However, the U-LTE$_4$ concentration significantly increases in patients with not only eosinophilic vasculitides, including CSS, but also noneosinophilic vasculitides (Fig. 3).[14]

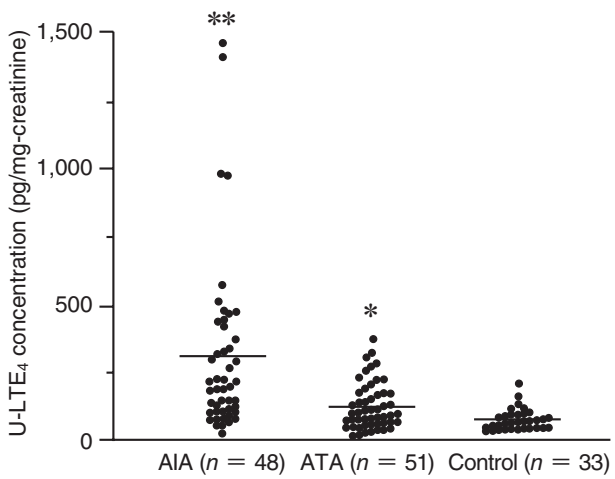

Fig. 1 Basal levels of urinary LTE$_4$ in patients with aspirin-intolerant asthma (AIA) and patients with aspirin-tolerant asthma (ATA). Horizontal bars indicate geometric means. * $p < 0.001$ compared with the control group; * * $p < 0.0001$ compared with the ATA group. (Adapted from Kawagishi *et al.*[12])

CysLTs OVERPRODUCTION IN ACUTE ASTHMA

1) SPONTANEOUS ATTACK

In acute asthmatics, the urinary excretion of LTE$_4$ was significantly higher on admission with an asthma attack, and returned to control levels when the patient's conditions improved. However, the increased level of U-LTE$_4$ was only 2-fold higher than that of healthy controls (Fig. 4).[15] Oosaki also reported that there is a significant correlation between changes (%) in urinary eosinophil protein X (EPX), which may originate from eosinophil activation, and those in urinary LTE$_4$ during the spontaneous attack state.[16] Moreover, Green *et al*. reported that the decreases in FEV$_1$ are significantly correlated to U-LTE$_4$ concentrations in patients with acute exacerbation of asthma.[17] Tanaka and his colleagues found that the U-LTE$_4$ concentration at night significantly increased in

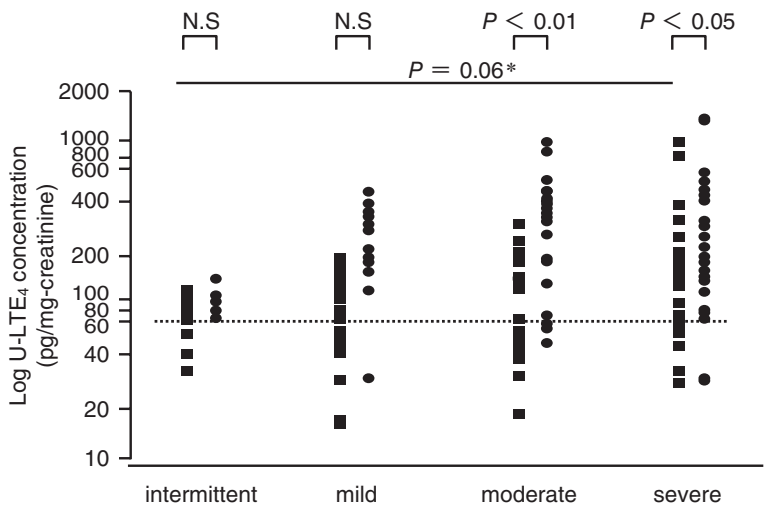

Fig. 2 Urinary LTE$_4$ concentration in patients with aspirin-intolerant asthma (AIA) and patients with aspirin-tolerant asthma (ATA) classified according to clinical severity of asthma. U-LTE$_4$ concentration is expressed by using the log scale. Patients with ATA and patients with AIA are denoted by closed square (■) and closed circle (●), respectively. The dotted line indicates the mean level of U-LTE$_4$ in healthy control subjects. *U-LTE$_4$ concentration in patients with ATA with different clinical asthma severity levels were compared by using the Kruskal-Wallis test. (Adapted from Higashi *et al.*[11])

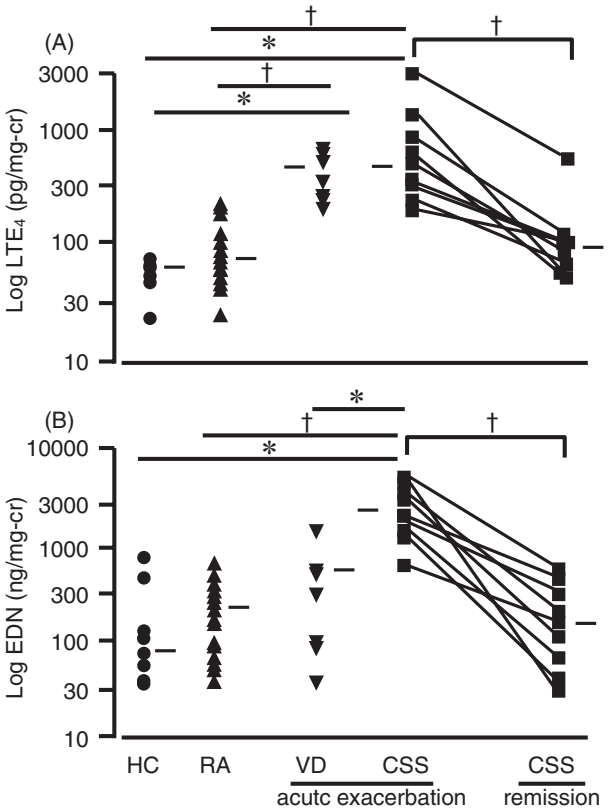

Fig. 3 Urinary LTE_4 (**A**) and EDN (**B**) concentration in each group. Urinary concentrations are expressed by using the log scale. Horizontal bars indicate medians. $*p < 0.05$; $\dagger p < 0.01$. HC: healthy control, RA: rheumatoid arthritis, VD: non-eosinophilic vasculitis, CSS: Churg-Strauss syndrome. (Adapted from Higashi *et al.*[14])

patients with nocturnal asthma.[18] These findings suggest that systemic CysLTs overproduction during a spontaneous attack is mainly caused by eosinophils, and that it is closely related to human airflow limitation.

2) ALLERGEN-INDUCED ASTHMATIC REACTION

The urinary LTE_4 concentration increases during the first 3h after allergen inhalation in atopic patients.[19,20] However, no significant increase is observed in the late phase during allergen-induced bronchoconstriction (Fig. 5).[19] Moreover overproduction of CysLTs in allergen-induced asthma was found to be not associated with an increased concentration of LTB_4 glucuronide in urine.[19] Very recently, we have quantified lipid mediators in exhaled breath condensate (EBC) and their corresponding urinary metabolites before and after allergen inhalation. In the patients with allergen-induced early asthmatic responses (EARs), the decreased percentage in FEV_1 significantly correlated with the increase in CysLTs concentration in EBC but not with the increase in U-LTE_4 concentration after allergen inhalation (Fig. 6).[21] There was a significant correlation between increased concentrations of CysLTs and PGD_2 in EBC collected in patients with EARs after allergen inhalation. However, the increase in urinary 9α, 11β-PGF_2 (metabolite presumably related to mast cell activation.[22]), concentrations did not correlate with either the increase in PGD_2 concentration in EBC or that in LTE_4 concentration in urine.[21] These results suggest that (1) human EARs may be mainly induced by CysLTs generated by pulmonary mast cells and (2) urinary LTE_4

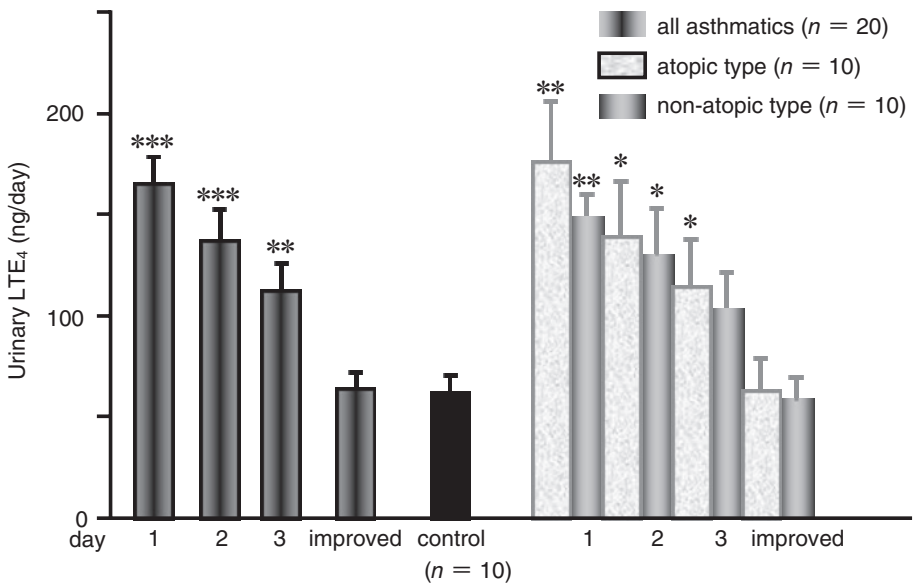

Fig. 4 Change in urinary LTE_4 concentration before and after treatment in patients with the spontaneous asthma attack. $* p < 0.05$; $** p < 0.01$; $*** p < 0.01$ compared with improved state. (Adapted from Oosaki *et al.*[15])

and PGD_2 metabolite may not be direct indicators of eicosanoid production in the airway.

HYPERLEUKOTRIENURIA IN SEASONAL ALLERGIC RHINITIS (SAR)

CysLTs have been reported to play a primary role in the induction of nasal blockage associated with allergic rhinitis, with limited effect on other rhinitis symptoms.[23-25] We demonstrated for the first time that the basal U-LTE_4 concentration is significantly higher in SAR patients with severe nasal blockage than in those with mild or no nasal blockage and in healthy control subjects.[26] More interestingly, there was no significant difference in the U-LTE_4 concentration between the patients with both SAR and asthma and SAR patients with severe nasal blockage (Fig. 7).[26] There is a significant correlation between U-LTE_4 and 9α11 βPGF_2 concentrations. On the other hand, there is no significant correlation between U-LTE_4 and eosinophil-derived neurotoxin (EDN) concentrations.[26] These results indicate that nasal blockage may be induced by the overproduction of CysLTs generated by mast cells rather than by eosinophils in the nasal cavity of SAR patients.

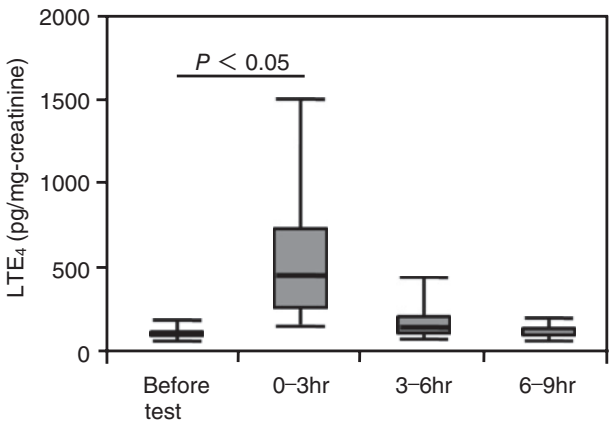

Fig. 5 Change in urinary LTE_4 concentration before and after allergen inhalation in patients with mite-sensitive asthmatics. (Adapted from Mita *et al.*[19])

ROLE OF CysLTs IN THE PATHOGENESIS OF NASAL POLYPOSIS

Nasal polyps are edematous semitranslucent masses in the nasal and paranasal cavities, mostly originating from the mucosal linings of the sinuses and prolaps-

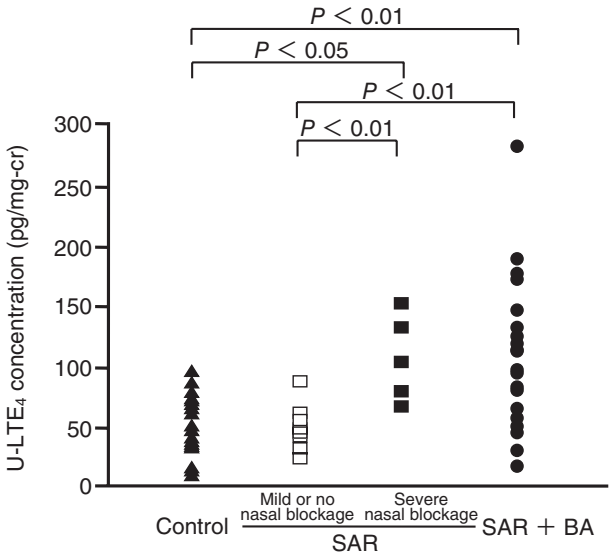

Fig. 7 Urinary leukotriene E_4 concentration (pg/mg of creatinine) in each group. Healthy control subjects, seasonal allergic rhinitis patients with mild or no nasal blockage, seasonal allergic rhinitis patients with severe nasal blockage, and patients with both seasonal allergic rhinitis (SAR) and asthma (BA). (Adapted from Higashi *et al.*[26])

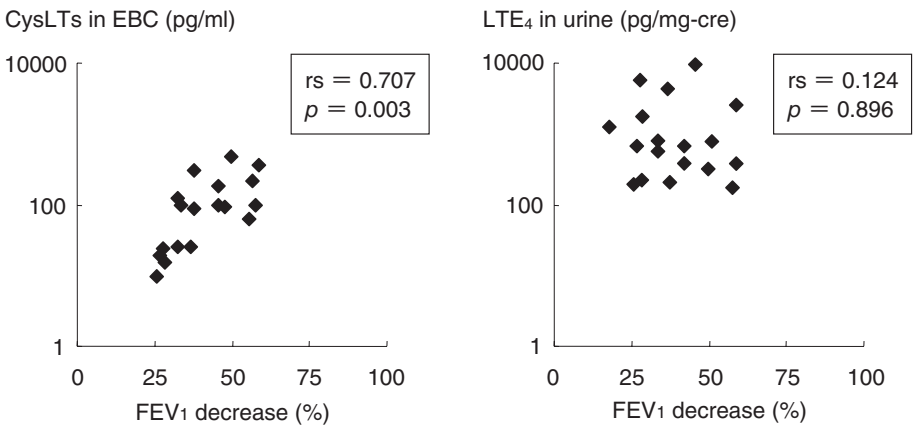

Fig. 6 In the patients with allergen-induced early asthmatic responses (EARs), the decreased percentage in FEV_1 significantly correlated with the increase in CysLT concentration in EBC (**A**) but not with the increase of Urinary LTE_4 concentration (**B**) after allergen inhalation. (Adapted from Ono *et al.*[21])

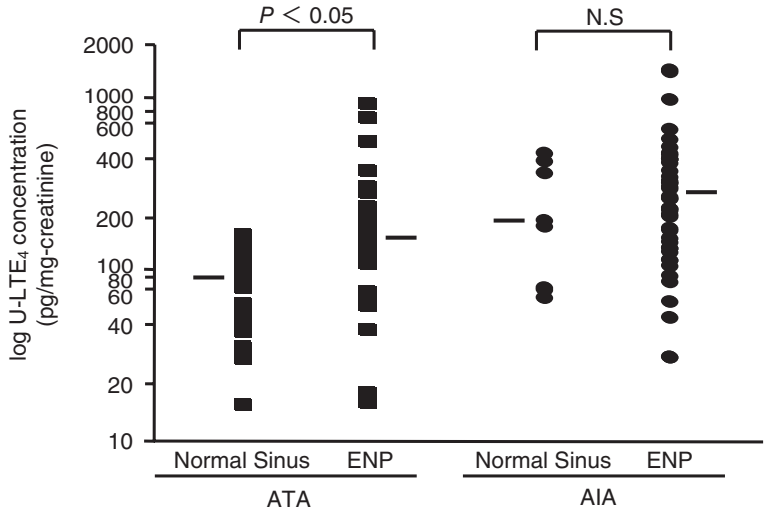

Fig. 8 Urinary LTE_4 concentration in aspirin-tolerant asthma (ATA) and aspirin-intolerant asthma (AIA) patients with eosinophilic nasal polyposis (ENP) and those with normal sinuses. Urinary LTE_4 concentration is expressed by using the log scale. The horizontal bars indicate medians. (Adapted from Higashi *et al.*[11])

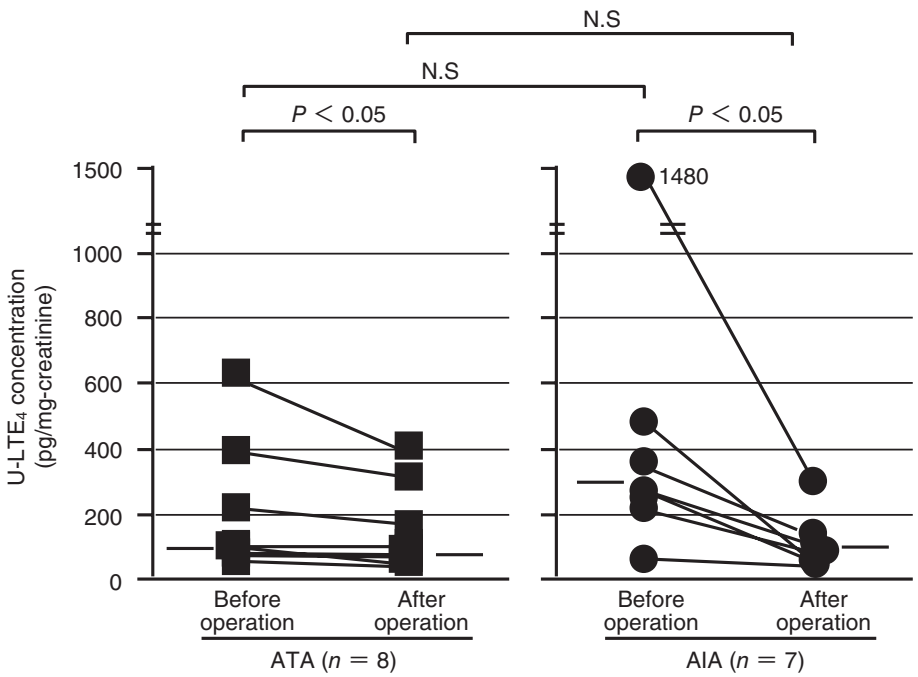

Fig. 9 A significant decrease in urinary LTE_4 concentration between before and after the endoscopic sinus surgery without changing medication. The horizontal bars indicate medians. (Adapted from Higashi *et al.*[11])

ing into the nasal cavities. Eosinophilic nasal polyposis (ENP), in which there is prominent infiltration of eosinophils and submucosal edema, is linked to comorbidities such as nonatopic asthma, adult-onset asthma, and aspirin intolerance, or may represent a part of a systemic disease such as CSS. Despite sinus surgery or intensive steroid therapy, the recurrence rate of ENP in patients with asthma is very high, and the pathogenesis of ENP has not been clarified.[27]

For the first time, we found that the U-LTE_4 concentration is significantly high even in ENP patients without aspirin sensitivity (Fig. 8).[11] We then confirmed the changes in U-LTE_4 concentration after endoscopic sinus surgery without changing the medica-

tion. We demonstrated that U-LTE4 concentrations significantly decreased after the sinus surgery in both AIA and ATA patients (Fig. 9).[11] Steinke *et al*. reported that the CysLTs concentrations in ENP tissues are significantly higher than those in noneosinophilic nasal polyp tissues.[28] They also demonstrated that the presence of CysLTs in ENP tissues is associated with an increased expression level of LTC4syntase mRNA.[28] Perez-Novo *et al*. confirmed that the concentrations of CysLTs and eosinophil cationic protein (ECP) and the expression level of LTC4synthase mRNA in ENP tissues are significantly higher than those in tissues with sinusitis without polyps.[29] From this observation, we conclude that ENP tissues contain and produce large amounts of CysLTs, and that there is a close correlation between CysLT production and eosinophil accumulation in ENP tissue.[30] CysLTs have several inflammatory effects on eosinophils, which are not only activation, but also an effect on trafficking and apoptosis inhibition.[31-33]

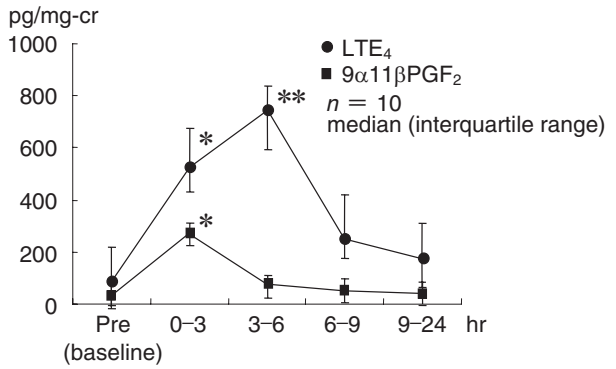

Fig. 10 Changes in urinary LTE_4 and 9α, 11β-PGF_2 concentrations in anaphylactic patients after provocation test. * $p < 0.01$, ** $p < 0.001$ compared with baseline. (Adapted from Ono *et al.*[37])

CysLT OVERPRODUCTION IN HUMAN ANAPHYLAXIS

Anaphylaxis is a life-threatening generalized allergic reaction that occurs when antigens bind to immunoglobulin E on mast cells and basophiles causing the release of inflammatory mediators.[34,35] We focused on the changes in inflammatory mediator concentrations during anaphylactic reactions in our recent study. We have demonstrated that anaphylaxis is associated with increased excretions of LTE_4 and 9α, 11 β-PGF_2 (Fig. 10),[36,37] and a significant correlation between the concentrations of these two mediators.[37] The observations suggest that mast cells may participate in the generation of CysLTs during the anaphylactic reactions. The changes in mediator concentrations may provide valuable information on therapeu-

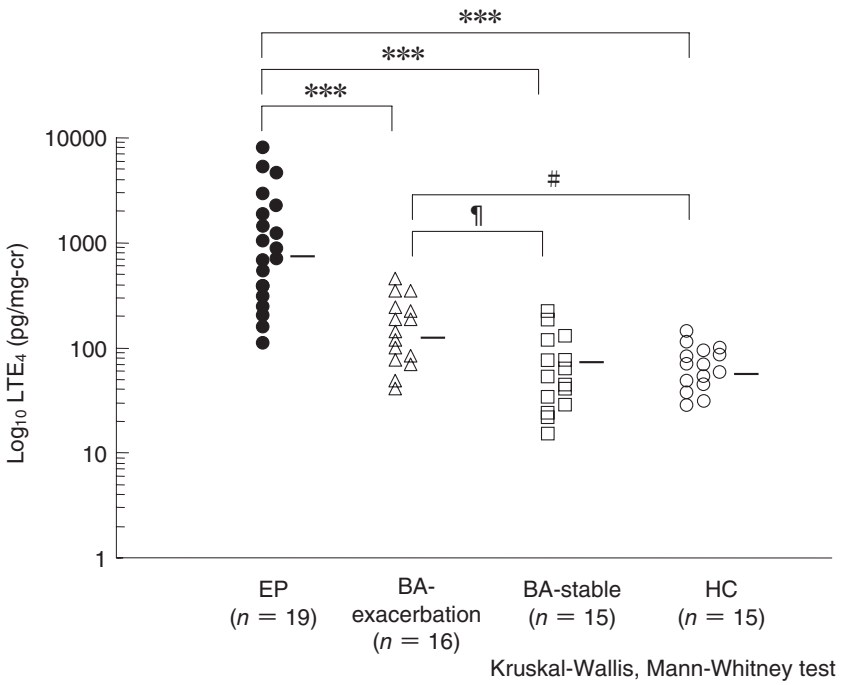

Fig. 11 Urinary LTE4 concentrations in 19 eosinophilic pneumonia (EP) subjects, 18 patients with exacerbations of bronchial asthma (BA), 15 patients with stable BA and 15 healthy controls (HC). Horizontal bars indicate medians. cr: creatinine. #: $p = 0.036$; ¶: $p = 0.042$; ***: $p < 0.001$. (Adapted from Ono *et al.*[41])

tic targets in anaphylaxis.

EOSINOPHILIC PNEUMONIA (EP) AND CysLTs OVERPRODUCTION

Eosinophilic pneumonia (EP) is a diffuse infiltrative lung disease characterized by alveolar and peripheral airway eosinophilia.[38-40] Although eosinophils produce cysteinyl leukotrienes (CysLTs) in large quantities, information on the relationship between CysLTs and eosinophilic pneumonia (EP) is lacking. We demonstrated that the urinary LTE_4 concentration is significantly higher in EP patients during acute exacerbation than in asthma patients with acute exacerbation and healthy subjects (Fig. 11),[41] and the concentration significantly decreases in EP patients during clinical remission. These findings suggest that CysLTs production is closely associated with the clinical conditions of EP patients. We also demonstrated that the progression of eosinophilic pneumonia is associated with elevated urinary leukotriene E_4 and eosinophil-derived neurotoxin concentrations, which may originate from eosinophil activation.[41] The leukotriene E_4 concentration was correlated with the level of diffusing capacity of the lung for carbon monoxide during acute exacerbation.[41] These findings suggest that the monitoring of the leukotriene E_4 concentration may aid in the management of eosinophilic pneumonia patients.

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